SUPPLEMENTAL MATERIAL

Human memory $CD4^+$ T cell immune responses against *Giardia lamblia*

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Table S1. *Giardia* A (SSA) and B (SSB) antigen extract induced cytokine production in effector memory CD4$^+$ T cells after 24 hours and, surface activation markers and proliferation after 144 hours (all values are in ‰ positive effector memory cells/all effector memory cells or ‰ CD4$^+$ T cells/all CD4$^+$ T cells (median (SD)) in the study populations).

	SSA responses				SSB responses			
Cytokines, T cell activation markers and proliferation	Low risk controls[1]	**Recent** giardiasis[2]	**Current** giardiasis[3]	**Combined** *Giardia* exposed[4]	Low risk controls[1]	**Recen**t giardiasis[2]	**Current** giardiasis[3]	**Combined** *Giardia* exposed[4]
24 hours								
IL-17A	0 (0)	0 (0.35)	0.88 (0.50)	0 (0.47)	0.17 (1.04)	0.52 (0.97)	1.58 (2.74)	0.65(1.65)
IFN-γ	0.29 (0.20)	0 (0.97)	0 (0.59)	0 (0.88)	0.19 (2.16)	0.36 (1.39)	0.60 (0.35)	0.39 (1.23)
TNF-α	1.22 (5.48)	1.99 (4.15)	4.81 (5.81)	2.6 (4.74)	0 (0.0)	0 (0.23)	0.67 (0.32)	0 (0.42)
IL-17A and IFN-γ	0 (0.02)	0 (0.04)	0.07 (0.13)	0 (0.08)	0.13 (0.83)	0.19 (0.44)	1.10 (1.59)	0.27 (0.91)
IL-17A and TNF-α	0.12 (0.55)	0.60 (0.94)	3.58 (4.14)	0.70 (2.58)	0 (0)	0 (0.12)	0 (0.04)	0 (0.11)
IFN-γ and TNF-α	0.33 (1.61)	0.24 (1.91)	1 (1.17)	0.29 (1.74)	0 (0.02)	0 (0.03)	0.13 (0.16)	0 (0.09)
IL-17A, TNF-α and IFN-γ	0.10 (0.46)	0.25 (0.56)	2.1 (1.87)	0.28 (1.2)	0 (0)	0 (0.07)	0 (0.02)	0. (0.06)
144 hours								
CD45RO and HLA-DR	0.7 (12.1)	35.5 (70.7)	14.9 (51.3)	30.5 (66.2)	1.9 (4.5)	16.3 (74.2)	30.1 (12.9)	24.1 (65.5)
CD45RO/HLA-DR and CD25/CD26	0.1 (2.4)	3.5 (26.9)	1.4 (11.6)	2.9 (24.2)	0.2 (0.7)	2.0 (11.3)	2.2 (1.6)	2.1 (10.2)
Proliferation	4.3 (46.6)	95 (118)	27.1 (149)	58.0 (120)	5.1 (14.6)	22.5 (112)	51.1 (14.3)	33.6 (98.4)
CD45RO/HLA-DR and proliferation	0.3 (12)	30.4 (70.7)	13.7 (50)	25.0 (66.0)	0.8 (4.1)	13.2 (74.4)	29.7 (11.4)	21 (65.5)

[1]N=12, [2]N=16, [3]N=5, [4]N=21 (N=11 for low risk controls in the cytokine assay. In the activation markers and proliferation markers assay N=18 for combined *Giardia* exposed, N=14 for recent giardiasis individuals and N=4 for current giardiasis individuals).

Table S2. Strenghts of association for cytokine production, surface activation markers and proliferation induced by *Giardia* A (SSA) and B (SSB) antigen extracts in a combined analysis of low risk controls and the two subgroups with recent or ongoing giardiasis. Kruskal-Wallis non-parametric comparison was first done for all three groups. If the p-value was below 0.05, Mann-Whitney test was done for 2-group comparisons.

	SSA stimulation				SSB stimulation			
Cytokines, T cell activation markers and proliferation	Three group comparison: Kruskal-Wallis test	Low risk controls[1] vs **recent** giardiasis group[2]	Low risk controls[1] vs **current** giardiasis group[2]	**Recent** giardiasis group[2] vs **current** giardiasis group[3]	Three group comparison: Kruskal-Wallis test	Low risk controls[1] vs **recent** giardiasis group[2]	Low risk controls[1] vs **current** giardiasis group[2]	**Recent** giardiasis group[2] vs **current** giardiasis group[3]
24 hours								
IL-17A	0.002	-	0.001	0.011	0.030	-	0.019	-
IFN-γ	-	-	-	-	-	-	-	-
TNF-α	-	-	-	-	-	-	-	-
IL-17A and IFN-γ	-	-	-	-	-	-	-	-
IL-17A and TNF-α	0.005	0.048	0.006	0.017	-	-	-	-
IFN-γ and TNF-α	-	-	-	-	-	-	-	-
IL-17A, TNF-α and IFN-γ	-	-	-	-	-	-	-	-
144 hours								
CD45RO and HLA-DR	0.024	0.009	-	-	0.015	0.029	0.008	-
CD45RO/HLA-DR and CD25/CD26	0.048	0.015	-	-	0.034	0.023	0.039	-
Proliferation	-	-	-	-	0.017	0.040	0.008	-
CD45RO/HLA-DR and proliferation	0.024	0.011	-	-	0.004	0.006	0.005	-

[1]N=12,[2]N=16, [3]N=5 (N=11 for low risk controls in the cytokine assay. In the activation markers and proliferation markers assay, N=14 for recent giardiasis individuals and N=4 for current giardiasis individuals).

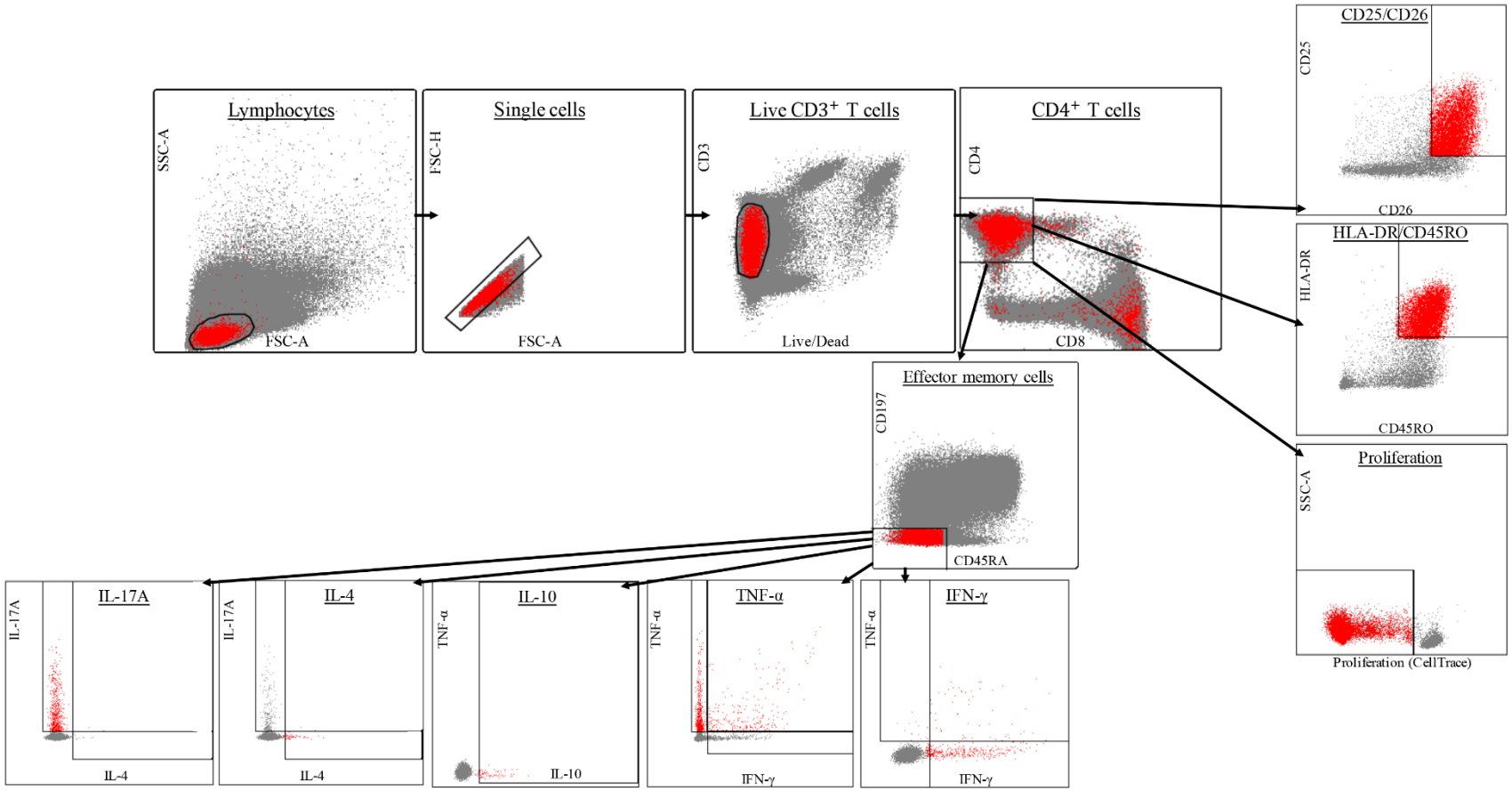

Figure S1. Gating strategy for the flow cytometric analysis. Lymphocytes were first gated and doublets and triples removed by single cell gating. Live $CD3^+$ T cells were gated by excluding dead cells/$CD14^+$ cells. $CD4^+$ T cells were separated from $CD8^+$ T cells. For the flow cytometric cytokine analysis done after 24 hours cell stimulation, effector memory $CD4^+$ T cells were gated and cytokine production was analyzed. The flow cytometric proliferation and activation markers measured after six days cell stimulation, were gated from the general $CD4^+$ T cell population. The red color inside the gates from the lymphocyte population to the $CD4^+$ T cell population represents cell population density. The red color of the subpopulations, represents the positive cells.